BEHAVIOR OF SOME STRAINS OF *YERSINIA ENTEROCOLITICA* AT FREEZING AND SALT

C. CUMPĂNĂŞOIU[1], C.E. CUMPĂNĂŞOIU[2], R. TRIF[1], E. TÎRZIU[1], ILEANA NICHITA[1], MONICA ŞEREŞ[1], R.V. GROS[1], BIANCA CUMPĂNĂŞOIU[1]

[1]Faculty of Veterinary Medicine Timisoara, 300645, Calea Aradului No. 119, Timisoara, Romania
[2]DSVSA Ramnicu Valcea, Timisoara, România
E-mail: ciceronis56@yahoo.com

Summary

Food can be a risk factor for involvement in a number of pathological conditions. Achieve healthy food is a necessity and therefore rules to control food hygiene were introduced. The present research followed the tolerance of *Yersinia enterocolitica* strains isolated from pigs' faeces at some variations of temperature and sodium chloride action. Experiments were conducted on five strains of *Yersinia enterocolitica* obtained after processing of 30 samples collected directly from the pigs' rectum. The results obtained confirm the sensitivity of bacteria at low temperatures and hyper-salt media.

Key words: *Yersinia enterocolitica*, tolerance, temperature, NaCl

Yersinia enterocolitica is a widespread bacteria and has been isolated from the intestinal tract of many domestic and wild mammalian species, but also rodents, birds, fish, frogs, molluscs, crustaceans and humans. A large number of researchers argue that *Yersinia enterocolitica* is a pathogen that can contaminate most food products, among which we mention pork, beef and lamb meat, milk and milk products, especially raw milk, pasteurized milk, milk powder, cream and ice cream, vegetables, seafood etc. (1, 2, 5).

Given this, method of conservation is essential to avoid contamination of food. Thus, although freezing is considered crucial given, numerous national and international literature states that low temperatures play a role in starting the process of meat' alteration. As is known, microorganisms do not replicate at frozen temperature and gradually lose their viability while enzymatic systems are relatively resistant and remain active even at a negative temperature of -30°C (3, 4, 6, 7).

Materials and methods

The research was conducted on a number of five *Y. enterocolitica* strains, obtained from pigs. For isolation and identification of *Y. enterocolitica* strains, a total of 30 samples were initially taken from the rectum of the pigs, using cotton swabs. The samples were first seeded in the usual and enriched media (PSB and ITC) and then on selective media. Strains were identified as *Y. enterocolitica*

species, were subjected to chemical tests, after which were reseeded in broth to study behavior at freezing, refrigeration, and in presence of NaCl.

Results and discussions

Study of thermal factor action on the development, namely inhibition of *Y. enterocolitica* bacterial populations at freezing and refrigeration temperatures, was performed on five strains which were seeded in liquid medium (meat broth) and exposed to temperatures between -18°C to + 4°C for a period of 2-30 days (Table 1).

Table 1

Experimental scheme

No. of strains	No. of cells/ml culture	Specification		
		Temperature (°C)	Period (days)	No. of samples
5	10^6	- 18	20	5
			30	5
		- 12	20	5
			30	5
		- 4	20	5
			30	5
		0	2	5
			4	5
		+ 4	2	5
			4	5

The results obtained showed that at 4°C the number of viable cells is maintained, as the increase of freezing temperatures, to register a significant decrease (from 10^6 to 10^2). At refrigeration temperature strains remain viable but their number was reduced at 0°C comparing to 4°C, temperature at which there is a slow multiplication (Table 2).

We also mention the important role of exposure to low temperatures. For example, in samples kept 20 days at -18°C and maintained 30 days at the same temperature, viability differences ranged from one to three logarithms. Analyzing the data we can say that if the exposure period was lower (eg, two and four days), difference in *Y. enterocolitica* strains viability was almost insignificant. In the samples maintained under refrigeration for a longer period of time a logarithmic growth of strains was observed (Fig. 1).

Thus, we conclude that increases of the exposure to freezing temperatures (-18 °) reduced the number of viable cells, while refrigeration temperature has increased them, but different from one strain to another. Most constant values were recorded for strains 1 and 2. The results are similar to those published in literature (4).

Table 2

Tolerance of *Y. enterocolitica* at freezing and refrigeration temperatures

Strains	Cells/ml culture	No. of sample	Temperature									
			-18°C		-12°C		-4°C		0°C		4°C	
			20 days	30 days	20 days	30 days	20 days	30 days	2 days	4 days	2 days	4 days
1	**10^6**	1	10^5	10^5	10^5	10^4	10^5	10^4	10^5	10^5	10^4	10^5
		2	10^5	10^5	10^5	10^4	10^5	10^4	10^5	10^4	10^5	10^6
		3	10^5	10^5	10^4	10^3	10^4	10^3	10^4	10^4	10^4	10^5
		4	10^5	10^5	10^5	10^4	10^5	10^4	10^5	10^5	10^4	10^4
		average	**10^5**	**10^5**	**$10^{4,75}$**	**$10^{3,75}$**	**$10^{4,75}$**	**$10^{3,75}$**	**$10^{4,75}$**	**$10^{4,5}$**	**$10^{4,25}$**	**10^5**
2	**10^6**	1	10^5	10^4	10^5	10^4	10^4	10^3	10^4	10^6	10^4	10^5
		2	10^5	10^4	10^4	10^3	10^5	10^5	10^4	10^5	10^4	10^6
		3	10^5	10^4	10^4	10^4	10^4	10^3	10^4	10^4	10^4	10^5
		4	10^5	10^4	10^5	10^4	10^5	10^4	10^5	10^5	10^4	10^4
		average	**10^5**	**10^4**	**$10^{4,5}$**	**$10^{3,75}$**	**$10^{4,5}$**	**$10^{3,75}$**	**$10^{4,25}$**	**10^5**	**10^4**	**10^5**
3	**10^6**	1	10^4	10^2	10^4	10^2	10^5	10^3	10^3	10^5	10^3	10^5
		2	10^4	10^2	10^4	10^2	10^5	10^2	10^4	10^5	10^4	10^6
		3	10^4	10^2	10^4	10^2	10^4	10^3	10^3	10^4	10^4	10^5
		4	10^5	10^3	10^3	10^2	10^4	10^4	10^3	10^5	10^4	10^4
		average	**$10^{4,25}$**	**$10^{2,25}$**	**$10^{3,75}$**	**10^2**	**$10^{4,5}$**	**10^3**	**$10^{3,25}$**	**$10^{4,75}$**	**$10^{3,75}$**	**10^5**
4	**10^6**	1	10^5	10^2	10^4	10^2	10^5	10^5	10^5	10^5	10^5	10^5
		2	10^5	10^2	10^4	10^2	10^5	10^4	10^5	10^4	10^5	10^6
		3	10^5	10^2	10^4	10^2	10^4	10^4	10^4	10^4	10^4	10^5
		4	10^5	10^2	10^4	10^2	10^5	10^5	10^5	10^5	10^4	10^4
		average	**10^5**	**10^2**	**10^4**	**10^2**	**$10^{4,75}$**	**$10^{4,5}$**	**$10^{4,75}$**	**$10^{4,5}$**	**$10^{4,5}$**	**10^5**
5	**10^6**	1	10^5	10^4	10^5	10^5	10^5	10^3	10^5	10^6	10^4	10^5
		2	10^5	10^4	10^5	10^4	10^5	10^4	10^4	10^5	10^4	10^6
		3	10^6	10^5	10^5	10^4	10^4	10^3	10^4	10^4	10^4	10^5
		4	10^5	10^4	10^4	10^3	10^5	10^4	10^5	10^5	10^4	10^4
		average	**$10^{5,25}$**	**$10^{4,25}$**	**$10^{4,75}$**	**10^4**	**$10^{4,75}$**	**$10^{3,5}$**	**$10^{4,5}$**	**10^5**	**10^4**	**10^5**

Also it was studied the effect of salt on the *Y. enterocolitica* bacterial populations in a liquid medium (beef broth), to which were added different concentrations of salt (Table 3).

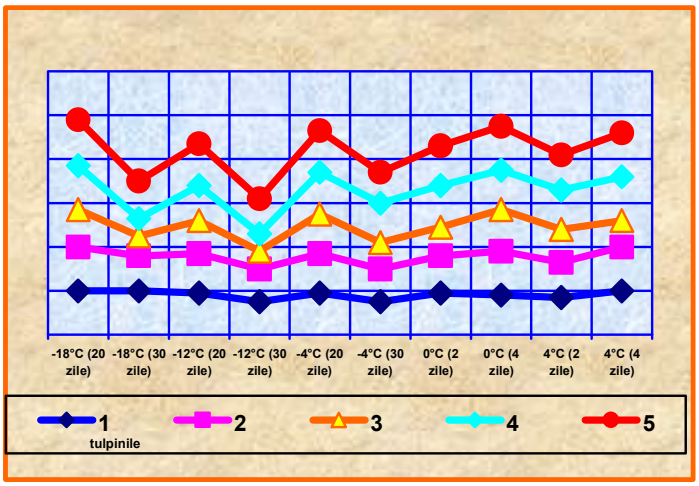

Fig. 1. *Y. enterocolitica* strains behavior at freezing and refrigeration temperatures

The results showed that *Y. enterocolitica* strains are tolerant at 5% NaCl, over this percentage occurring death culture. For example, after exposure to a 7% salt concentration for 72 hours, we fail to recover strains by passage in nutrient broth. Note that evolution of the strains studied was different depending on the salt concentration in the culture medium (Fig. 2).

Table 3

***Y. enterocolitica* strains tolerance to salt action**

Strain	Cells/ml	No. of sample	NaCl concentration, exposure for 72 h					
			0%	0,5%	2,5%	4%	5%	7%
1	10^5	1	10^4	10^4	10^6	10^5	10^4	0
		2	10^5	10^5	10^7	10^6	10^5	0
		3	10^3	10^3	10^5	10^4	10^3	0
		4	10^4	10^4	10^6	10^5	10^4	0
		average	**10^4**	**10^4**	**10^6**	**10^5**	**10^4**	**0**
2	10^5	1	10^4	10^4	10^6	10^5	10^4	0
		2	10^5	10^5	10^7	10^6	10^5	0
		3	10^3	10^3	10^5	10^4	10^3	0
		4	10^4	10^4	10^6	10^5	10^4	0
		average	**10^3**	**10^3**	**10^5**	**10^4**	**10^3**	**0**
3	10^5	1	10^4	10^4	10^6	10^5	10^4	0
		2	10^5	10^5	10^7	10^6	10^5	0
		3	10^3	10^3	10^5	10^4	10^3	0
		4	10^4	10^4	10^6	10^5	10^4	0
		average	**10^3**	**10^3**	**10^4**	**10^4**	**10^3**	**0**

4	10^5	1	10^4	10^4	10^5	10^5	10^4	0
		2	10^5	10^3	10^5	10^6	10^3	0
		3	10^3	10^2	10^5	10^4	10^2	0
		4	10^4	10^3	10^5	10^5	10^3	0
		average	**10^4**	**10^3**	**10^5**	**10^4**	**10^3**	**0**
5	10^5	1	10^4	10^4	10^6	10^5	10^4	0
		2	10^5	10^5	10^7	10^6	10^5	0
		3	10^3	10^3	10^5	10^4	10^3	0
		4	10^4	10^4	10^6	10^5	10^4	0
		average	**10^4**	**10^4**	**10^6**	**10^4**	**10^3**	**0**

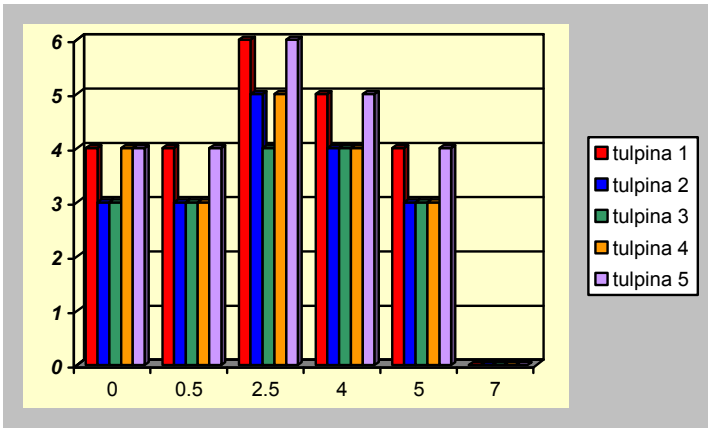

Fig. 2. Tolerance of *Yersinia enterocolitica* strains to salt action (average)

The maximum number of viable cells, compared with baseline, occurred in 2.5% and 4% salt. This confirms that the strains of *Y. enterocolitica* tolerate relatively high salt concentrations. So, increasing the salt concentration in the culture medium is inversely proportional to the concentration of pathogens (CFU / ml), reaching zero CFU / ml at 7% NaCl concentration and, as we mentioned, after 72 hours incubation at 37°C.

Conclusions

Yersinia enterocolitica is a bacteria sensitive to freezing, thus at the temperature of -18 ° C the number of *Y. enterocolitica* cells in pork meat is reduced by at least one log after 20 and 30 days.

We mention that at refrigeration temperatures (0°C and 2-4°C), *Y. enterocolitica* multiplied, multiplication being observed on raw pork, in which was registered the increase of the number of cells within a log, or even stationary.

It may be noted that in the event of massive contamination with these germs, period of storage is not always sufficient for providing healthy products for consumers. In conclusion, the authors propose to enhance bactericidal activity in this alimentary ring, such as freezing be assisted by ventilation to create a dry surface on the product.

As noted, the five serotypes of *Y. enterocolitica* were grown in the absence of salt and 2.5% NaCl concentration was optimal for the germs' development.

The concentration of 4% NaCl proved to be a limit of development, at 5% NaCl survival was still possible and at concentration of 7% NaCl was observed a total bactericidal effect on all strains studied.

It was noted that salt causes reduction in bacterial population when levels are above 5%. This aspect is helpful in guts' processing sectors of the slaughterhouses, where the processing of such products under hygienic conditions raises serious problems.

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